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Short communication

Determination of inositol hexanicotinate in rat plasma by high performance liquid chromatography with UV detection

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Abstract

A HPLC method with UV detection at 262 nm was developed to analyze inositol hexanicotinate in rat plasma. Plasma samples were extracted with an equal volume of acetonitrile, followed by dilution with mobile phase buffer (5 mM phosphate buffer, pH 6.0) to eliminate any solvent effects. Inositol hexanicotinate and the internal standard (mebendazole) were separated isocratically using a mobile phase of acetonitrile/phosphate buffer (35:65, v/v, pH 6.0) at a flow rate of 1.0 mL/min and a reverse-phase XTerra® MS C_{18} column (4.6 mm × 150 mm, 3.5 μm). The standard curve was linear over a concentration range of 1.5–100.0 μg/mL of inositol hexanicotinate in rat plasma. The HPLC method was validated with intra- and inter-day precisions of 1.55–4.30% and 2.69–21.5%, respectively. The intra- and inter-day biases were −0.75 to 19.8% and 2.58–22.0%, respectively. At plasma concentrations of 1.5–100 μg/mL, the mean recovery of inositol hexanicotinate was 99.6%. The results of a stability study indicated that inositol hexanicotinate was unstable in rat plasma samples, but was stable in acetonitrile extracts of rat plasma for up to 24 h at 4 °C. The assay is simple, rapid, specific, sensitive, and reproducible and has been used successfully to analyze inositol hexanicotinate plasma concentrations in a pharmacokinetic study using the rat as an animal model.

Keywords: Inositol hexanicotinate; HPLC; Plasma

1. Introduction

Inositol hexanicotinate, also known as no-flush niacin, is a niacin ester consisting of six molecules of niacin and one molecule of inositol [1,2]. Niacin has been widely used for decades for the treatment of hyperlipidemia. However, its use is often limited due to side effects such as skin flushing associated with immediate release niacin formulations, and hepatotoxicity associated with sustained release niacin formulations [3]. Inositol hexanicotinate has been used as a substitute for conventional niacin treatment [1]. The mechanisms of action of inositol hexanicotinate are believed to be the same as those for niacin, but inositol hexanicotinate is apparently free of acute side effects [4], which is thought to result from the sustained release of niacin *in vivo* through enzymatic hydrolysis of the ester [5]. However, recent clinical evaluations of the lipid-lowering effects of inositol hexanicotinate have raised concerns regarding its effectiveness [6–8]. The results of a study conducted in our laboratory revealed wide variations in the dissolution rates of twelve no-flush niacin products [9].

Inositol hexanicotinate products are sold as nutritional supplements which do not require a systematic FDA review and approval process. As a result, limited information exists in the literature on the *in vivo* absorption, distribution and metabolism of inositol hexanicotinate. Well-designed studies are needed to characterize the efficacy, safety, and pharmacokinetics of inositol hexanicotinate in order for pharmacists to better counsel patients regarding proper product selection.

Earlier studies on inositol hexanicotinate were focused on measuring its active metabolite, nicotinic acid [10–12]. Currently, no analytical method has been reported for the quantification of inositol hexanicotinate in plasma using HPLC. The purpose of this study was to develop a simple, rapid, specific, sensitive, and reproducible HPLC method for the analysis of

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inositol hexanicotinate in rat plasma. The assay was successfully used to analyze timed inositol hexanicotinate plasma concentrations in pharmacokinetic studies conducted in rats.

2. Experimental

2.1. Chemicals

Inositol hexanicotinate standard powder (98% pure) was purchased from Spectrum Chemical Mfg. Corp. (New Brunswick, NJ). The chemical structure of inositol hexanicotinate is presented in Fig. 1. Mebendazole and sodium phosphate were purchased from Sigma (St. Louis, MO). HPLC grade acetonitrile and methanol (Mallinckrodt Baker Inc., Phillipsburg, NJ) were used as received. Phosphoric acid and hydrochloric acid were obtained from Fisher Scientific (Fair Lawn, NJ). Deionized water was obtained using a Milli-Q analytical deionization system (Millipore, Bedford, MA).

2.2. Chromatography

The HPLC system (Waters Corporation, Milford, MA) consisted of a Waters 515 pump and a 717 plus autosampler. The system also included a Photodiode Array (PDA) detector monitoring at 262 nm, and a computer running Waters Empower 3.2 chromatography software. The mobile phase was composed of acetonitrile and 5 mM sodium phosphate buffer in a ratio of 35:65 (v/v). The final pH of the mobile phase was adjusted to 6.0 with phosphoric acid. The mobile phase was filtered and degassed through a 0.45 μm GHP membrane filter before use. Inositol hexanicotinate and mebendazole (internal standard, IS) were separated on a reverse-phase XTerra® MS C_{18} column (4.6 mm × 150 mm, 3.5 μm) equipped with an XTerra® MS C_{18} guard column (3.9 mm × 20 mm, 3.5 μm). The flow rate was set at 1.0 mL/min. The injection volume was 50 μL. Peak area ratios of inositol hexanicotinate to IS were employed for all calculations.

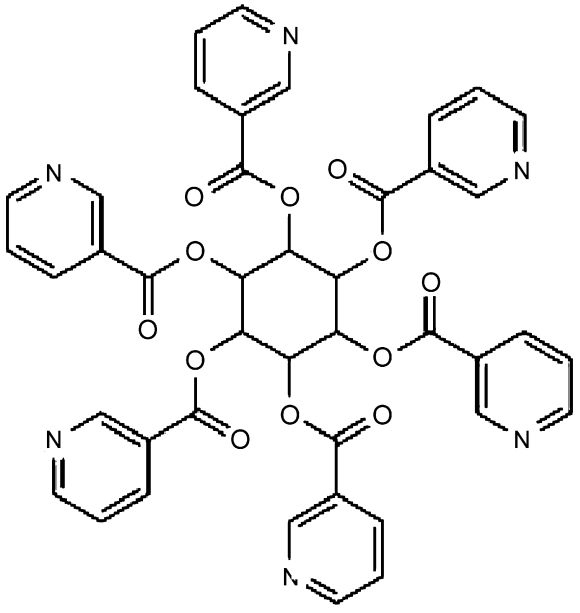

Fig. 1. Chemical structure of inositol hexanicotinate.

2.3. Standard solutions

An inositol hexanicotinate stock solution of 1 mg/mL was prepared in a 10-mL volumetric flask using methanol. For each stock preparation, 10 μL of concentrated hydrochloric acid was added to maintain drug solubility. A 1 mg/mL mebendazole internal standard stock solution was prepared in acetonitrile.

Fresh drug-free rat blood was collected from male Sprague–Dawley rats and the plasma obtained therefrom was stored at −80 °C prior to use. Blank rat plasma was spiked with inositol hexanicotinate to make 7 standard drug solutions (1.5, 2.5, 5, 10, 25, 50, and 100 μg/mL).

2.4. Sample extraction

An aliquot of plasma sample (100 μL) was placed in a 2-mL Eppendorf tube and the plasma was extracted and de-proteinized by mixing it with 100 μL of an acetonitrile solution containing 10 μg/mL of the IS. The mixture was briefly vortex-mixed for 15 s and centrifuged at 13,000 rpm for 8 min. An aliquot (100 μL) of the supernatant was transferred to another 2-mL Eppendorf tube and further diluted with 100 μL of the mobile phase buffer. The resultant solution was then vortex-mixed for 15 s and centrifuged at 13,000 rpm for 4 min. A 150 μL aliquot of the supernatant was transferred to an autosampler vial for HPLC analysis.

2.5. Assay validation

Linear calibration curves in rat plasma were generated by plotting the peak area ratio of inositol hexanicotinate to IS versus seven known inositol hexanicotinate concentrations. Slope, intercept and correlation coefficient values were estimated using least square regression analysis.

Validation of the HPLC method for inositol hexanicotinate in rat plasma was performed. Quality control plasma samples containing low, medium, and high inositol hexanicotinate concentrations were used to evaluate the precision and accuracy of the assay method. The intra-day assay precision and accuracy were obtained by analyzing the quality control samples in duplicate using a calibration curve constructed on the same day. The inter-day assay precision and accuracy were obtained analyzing the quality control samples in duplicate using calibration curves constructed on three different days. The assay precision was reflected by the relative standard deviation (R.S.D.%) and the assay accuracy was reflected by the relative percentage error from the theoretical drug concentrations.

The extraction recovery of inositol hexanicotinate from rat plasma was calculated as the ratio of the slope of a calibration curve for inositol hexanicotinate in spiked plasma to that in spiked mobile phase, and was expressed as a percentage. The limit of detection (LOD) was defined as the plasma concentration that yielded a peak height equal to three times

that of baseline noise. The limit of quantification (LOQ) was defined as the lowest concentration in the linear calibration curve.

2.6. Stability of inositol hexanicotinate in rat plasma

The stability of inositol hexanicotinate in rat plasma at room temperature and at −80 °C as a function of time was evaluated. The stability of inositol hexanicotinate in acetonitrile extracted supernatant pending HPLC injection was also evaluated. The peak area ratios from these samples were then compared to the ratios obtained from identical freshly prepared samples that were extracted and immediately analyzed.

3. Results

3.1. Chromatography

Fig. 2 shows representative chromatograms of blank plasma, blank plasma spiked with 25 μg/mL of inositol hexanicotinate and the IS, and a rat plasma sample obtained 5 min after a 50 mg/kg intravenous dose of inositol hexanicotinate. The chromatograms showed baseline separation of inositol hexanicotinate and the IS without any interference from endogenous plasma components. The retention times for inositol hexanicotinate and the IS were 6.1 and 7.3 min, respectively. The active metabolite, nicotinic acid, had a retention time of 1.7 min (data not shown).

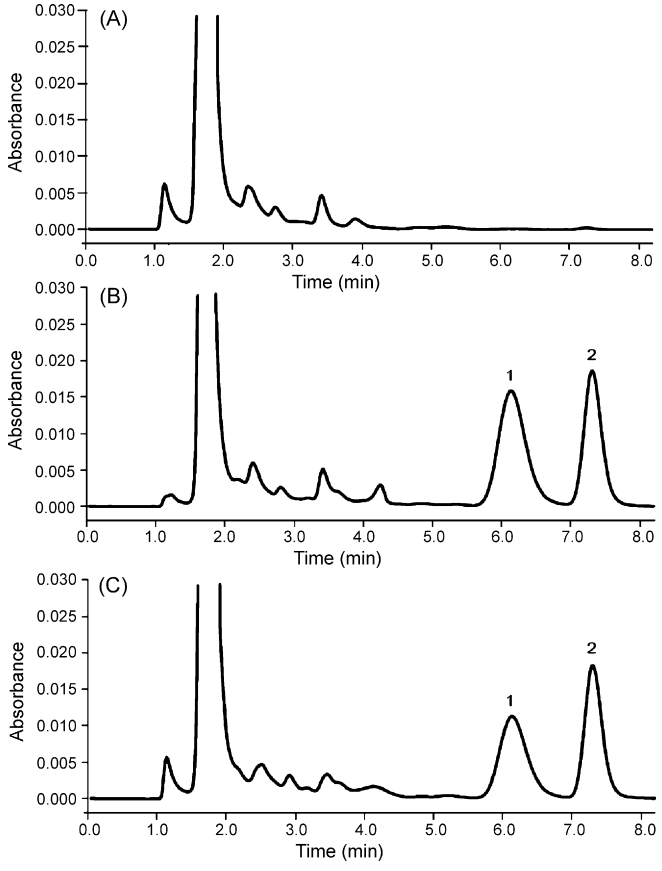

Fig. 2. Chromatograms of (A) blank rat plasma, (B) blank rat plasma spiked with 25 μg/mL inositol hexanicotinate and the internal standard mebendazole and (C) plasma sample from a rat 5 min after intravenous administration of a 50 mg/kg dose of inositol hexanicotinate. Peak #1 (6.1 min) is inositol hexanicotinate and Peak #2 (7.3 min) is mebendazole.

Table 1
Precision and accuracy of HPLC assay for inositol hexanicotinate in rat plasma

	Theoretical inositol hexanicotinate concentration		
	Low (2.5 μg/mL)	Medium (25 μg/mL)	High (75 μg/mL)
Intra-day ($n=6$)			
Mean	2.00	25.12	74.44
S.D.	0.086	0.497	1.151
R.S.D. (%)	4.3	1.98	1.55
Relative error (%)	19.8	0.496	−0.75
Inter-day ($n=30$)			
Mean	2.65	23.99	73.50
S.D.	0.57	1.08	1.98
R.S.D. (%)	21.5	4.50	2.69
Relative error (%)	22.0	4.87	2.58

3.2. Linearity, sensitivity and detection limit of the assay

The peak area ratio of inositol hexanicotinate to IS in rat plasma was linear with respect to inositol hexanicotinate concentration over the range of 1.5–100 μg/mL. The mean ($n=5$) linear regression equation for inositol hexanicotinate calibration curves was $y = 0.05427(\pm 0.00182)x - 0.0193(\pm 0.0447)$, where y is the peak area ratio of inositol hexanicotinate to IS and x is the concentration of inositol hexanicotinate in plasma. The correlation coefficient associated with all calibration curves for inositol hexanicotinate was above 0.999. The LOD and LOQ were 0.75 and 1.5 μg/mL, respectively.

3.3. Extraction recovery

At plasma concentrations of 1.5–100 μg/mL, the mean recovery of inositol hexanicotinate from plasma was 99.6 ± 0.45% ($n=5$).

3.4. Accuracy and precision of the assay

The summary data for the intra- and inter-day precision and accuracy of the assay method for inositol hexanicotinate in plasma are shown in Table 1. The accuracy values obtained from the intra- and inter-day studies were within acceptable limits (within 10% relative error) except for the 2.5 μg/mL concentration point. The higher variation in accuracy and poor precision at the low concentration point may be due to inherent instability of inositol hexanicotinate in plasma samples.

3.5. Stability

The results of the stability studies are summarized in Table 2. We observed inositol hexanicotinate to be unstable in rat plasma samples at room temperature (25 °C) and at −80 °C.

Table 2
Stability of inositol hexanicotinate under various conditions

Storage condition	Nominal concentration	Mean (±S.D.) percent of nominal concentration remaining ($n = 3$)
Room temperature	5 mg/mL in dilute HCl (pH 1.8)	
1 h		93.3 ± 2.4
2 h		83.6 ± 2.6
3 h		79.2 ± 1.3
4 °C temperature	5 mg/mL in dilute HCl (pH 1.8)	
1 h		98.4 ± 0.65
2 h		95.8 ± 0.85
4 h		94.6 ± 2.4
−80 °C temperature	5 mg/mL in dilute HCl (pH 1.8)	
24 h		106 ± 1.3
48 h		97.1 ± 2.3
72 h		93.5 ± 1.0
1 week		81.5 ± 2.0
Room temperature	10 μg/mL in rat plasma	
30 min		35.6 ± 4.6
−80 °C temperature	10 μg/mL in rat plasma	
2 h		62.5 ± 4.8
24 h		50.1 ± 1.7
Room temperature	10 μg/mL stored in acetonitrile extracts from rat plasma	
30 min		98.7 ± 1.2
1 h		99.5 ± 1.8
2 h		97.2 ± 0.9
4 °C temperature	10 μg/mL stored in acetonitrile extracts from rat plasma	
2 h		100 ± 5.1
4 h		96.6 ± 5.5
24 h		102 ± 3.3

For instance, more than 60% of spiked inositol hexanicotinate was lost at room temperature within 30 min following the addition of inositol hexanicotinate to rat plasma. However, inositol hexanicotinate was stable at 4 °C for up to 24 h after acetonitrile extraction from plasma. Therefore, for pharmacokinetic studies, plasma samples should be extracted with acetonitrile immediately after collection and analyzed within 24 h using a temperature controlled autosampler at 4 °C.

4. Discussion

Inositol hexanicotinate is insoluble in water and in some organic solvents, but is soluble in dilute acid. However, the drug is not stable in such an acidic medium (Table 2). In order to find an appropriate solvent for the preparation of a stock drug solution, we tested various organic solvents including methanol, acetonitrile, ethanol, dimethyl sulfoxide, acetone, and *N*,*N*-dimethylacetamide. We found that inositol hexanicotinate was most soluble in methanol and standard drug solutions prepared in this solvent stable for up to 1 week at −80 °C temperature.

The instability of inositol hexanicotinate in plasma is apparently due to ester hydrolysis [5]. The immediate addition of the extraction solvent acetonitrile to freshly collected plasma was found to increase drug stability during HPLC analysis for up to 24 h at 4 °C.

The LOQ for our assay (1.5 μg/mL) was demonstrated to be sufficient for pharmacokinetic studies of inositol hexanicotinate using the rat as an animal model [13].

5. Conclusions

A new validated HPLC method has been developed to assess inositol hexanicotinate concentrations in rat plasma. The intra- and inter-day precision and accuracy for the plasma samples were within the acceptable 10% limit. The correlation coefficient associated with the linear calibration curves for inositol hexanicotinate in plasma was >0.999. The LOQ (1.5 μg/mL) was suitable for measuring timed plasma concentrations in inositol hexanicotinate pharmacokinetic studies.

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